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Original Article

Frequency of Tuberculosis in Patients with Enlarged Lymph Nodes and its cost-effective first line of Diagnosis in our Country

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Abstract

Tuberculosis is the commonest infectious disease in the developing world. Tuberculous lymphadenitis is one of the most common form of extrapulmonary tuberculosis.Many diagnostic tests are devised for its detection including direct smear examination. This cross-sectional study was designed to determine the frequency of tuberculosis in lymphadenopathy of different sites and its rapid diagnosis by FNAC and ZN(Ziehl-Neelsen)staining of aspitation smears. A descriptive cross-sectional survey was done on 100 cases of enlarged lymphnodes on different site at two private tertiary medical college hospital of Dhaka. After reporting granulomatous inflammation on papanicolaou staining (PAP stain) of aspirates obtained by FNAC, one unstained slide from each cases was subjected to special stain like ZN, to determine the AFB positivity. Cases positive for AFB on ZN stain were noted down along with their frequency and percentages. Cervical lymph nodes were the most commonly involved site (76%). Forty One cases (56%) of AFB positive smears were reported in granulomatous inflammation. Most cases of AFB-positive smears were associated with caseation necrosis (92%). Male were affected more (63%) than female. Fine Needle Aspiration Cytology (FNAC) is a simple and rapid diagnostic technique, along with ZN staining, that increases the diagnostic accuracy of this technique. Because of early availability of results, simplicity, minimal trauma and complications, the aspiration cytology is now considered a valuable diagnostic aid and is part and parcel of a pathologist's repertoire.

Introduction

With a population of 150 million, Bangladesh ranks sixth among countries with a high tuberculosis(TB) burden. The estimated prevalence and incidence rates of all forms of tuberculosis were respectively 387 and 223 per 100 000 population, in 2007. TB control activities were further expanded by increasing the number of peripheral laboratories, sputum collection or smearing centres so that access to TB diagnostic services improved. The case-detection rate increased to 73% in 2007**(** The new smear positive case detection rate is 73% when calculated using the most recent national population figure which is 143 million).The reported treatment success rate has increased to 92% for the cohort of patients registered in 2006. The National TB Guidelines were updated bringing national policies in line with more recent international recommendations. A nationwide disease prevalence survey is being conducted to establish more accurate estimates of the prevalence of tuberculosis and to assess the trend of the epidemic in the country [(1).]

Tuberculosis of the lymph node (tuberculosis lymphadenitis) is the most common form of extrapulmonary tuberculosis. In developing countries where the incidence of TB is high, tuberculosis lymphadenitis is one of the most frequent causes of lymphadenopathy (30–52%) [(2).]

Historically, lymphnode tuberculosis(LTB) has been called the "King's evil" referring to the divine benediction which was presumed to be the treatment for it. It was also referred to as "scrofula" meaning "glandular swelling"(Latin) and "full necked sow"(French). Peripheral lymph nodes are most often affected and cervical involvement is the most[(3,4,5).]

Tuberculosis is the most common fatal infectious disease in the world: one-third of the world's population is currently infected and more than 1.5 million people die each year due to

tuberculosis. Tuberculosis causes the second highest mortality rates from an infectious disease worldwide, after human immunodeficiency virus. Currently, 30% of the world's population is estimated to be infected with the disease[6]. The main risk factors for tuberculosis include older age, lower socioeconomic status (via crowding, homelessness, poor nutrition, etc.), and HIV[7]. Other risk factors for the development of tuberculosis include smoking, alcohol consumption, shortage of food, and contact with TB patients[8].

Diagnosis of tuberculous lymphadenitis still faces many challenges, though there are many applied diagnostic tools. The diagnosis of extrapulmonary TB is difficult, especially when clinical presentation is suggestive but bacteriological proof is lacking. The diagnosis confirmed by acid-fast bacilli (AFB) using conventional microscopy is simple and rapid but lacks sensitivity whereas culture is more sensitive and specific but takes several weeks to get the results [9]. However, early diagnosis of infection is important before the start of antituberculosis chemotherapy. Clinical diagnosis is usually dependent on microscopic detection of *M. tuberculosis* using Ziehl-Neelsen (ZN) stain and mycobacterium culture[10]. Fluorescent stain (FS) has been proven to be superior to the Ziehl-Neelsen (ZN) stain, especially in paucibacillary cases [11,12]. Fine needle aspiration cytology is a quick, minimally invasive, and cost-effective technique for the diagnosis of granulomatous diseases, like tuberculosis [13].

The PCR is a sensitive and specific technique which is frequently introduced in the diagnosis of tuberculous lymphadenitis. Tests employing polymerase chain reaction for the specific detection of mycobacterium belonging to the *M. tuberculosis* complex [14]. Multiplication of tubercle bacilli in any site of the human body causes a specific type of inflammation, with formation of a characteristic granuloma [15].

Tuberculosis is still a public health problem in Bangladesh and is one of the ten major causes of death. Although, tuberculous lymphadenitis is one of the most common forms of extrapulmonary tuberculosis, very few studies on the histopathology of this condition have been done in Bangladesh.

Recently, fine needle aspiration cytology (FNAC) has been successfully adopted to diagnose such lesions. Fine Needle Aspiration Cytology (FNAC) is a simple, quick and inexpensive method that is used to sample superficial masses like enlarged lymph node and is usually performed in the outpatient clinic. It causes minimal trauma to the patient and carries virtually no risk of complications. It is a simple, easy technique and reports can be made available within an hour. The objective of this descriptive study was to see the frequency of various pathological conditions detected on FNAC in patients presenting with lymph node swellings coming to two private tertiary medical college hospital of Dhaka

Materials And Methods

This is a cross-sectional study to screen patients with enlarged lymph node for Mycobacterium tuberculosis. The study was conducted in two private tertiary medical college hospital of Dhaka, during the period from July 2010 to July 2011. One hundred patients with enlarged lymph nodes at various sites were subjected to FNAC .

Smears were made from aspirated material, fixed immediately in 75% alcohol and stained by Papanicolaou method . One extra unstained slide was smeared from aspirated material at the same time from each cases. Cases which were diagnosed granulomatous inflammation consistent with tuberculosis on the basis of the criteria of tuberculosis, that is aggregates of epithelioid cells forming a granuloma with or without caseous necrosis and sometime presence of multinucleated giant cells on FNAC, were further analysed using special stain like ZN (Ziehl Nelson's) staining. It would increase the diagnostic accuracy of this technique and help to differentiate between different infectious causes which can present with the same morphology. These smears were examined under the light microscope by a microbiologist. The findings of pap staining were categorized as epithelioid granuloma with caseous necrosis and epithelioid granuloma without caseous necrosis. The finding of ZN staining was labeled as positive for AFB or negative for AFB, when there is presence or absence of pink beaded, and stumpy rod shaped organisms, usually exacellularly, within or at the periphery of the granuloma . The detailed history of the patient i.e. age, sex, site and duration of involvement and other investigations performed, were recorded.

Results

One hundred patients of enlarged lymph nodes of different sites were taken. The commonest site of lymphadenopathy was the neck, the cervical group of lymph nodes, constituting 69 cases. Among the remaining cases, 22 cases were submandibular, 5 cases inguinal and 4 cases were axillary group of lymph nodes.The FNAC sites were shown in table-1.

Table 1:Distribution of study population by lymph node site

Sites of lesion	Number of cases	Percentage
Cervical	69	69
Submendibular	22	22
Inguinal	5	5
Axillary	4	4
Total	100	100

The cases were divided into five groups, on the basis of brief cytological criteria adopted for classification were as follows

1. a)Tuberculous lymphadenitis: revealed caseous necrotic material, epithelioid cells, lymphocytes and an occasional Langhans' giant cell (strong evidence) Figure (1a). b) Suggestive of tuberculous lymphadenitis: revealed epithelioid cells and lymphocytes but no caseous necrotic material (weak evidence).Figure (1b).

2. Non-specific lymphadenitis: showed a very cellular smear, without any necrosis. The cells consisted of lymphocytes at its different stages of maturation, histocytes, tingible body macrophages and plasma cells.

3. Pyogenic lymphadenitis: revealed predominantly polymorphonuclear leukocytes, necrotic material and other lymphoid cells.
4. Metastatic carcinoma: showed malignant epithelial cells, usually arranged in groups or cluster, along with other lymphoid cells.
5. Lymphoma: Non-Hodgkin's lymphoma showed a monocellular pattern, consisting of lymphoblasts or lymphocytes. Hodgkin's lymphoma showed a mix cell population with the characteristic Reedsternberg giant cell.

Table-2:Different types of FNAC findings on the basis of cytological criteria

FNAC findings	Number of cases
Tubercular	73
Non-specific	12
Pyogenic	5
Metastatic	6
Lymphoma	4
Total	100

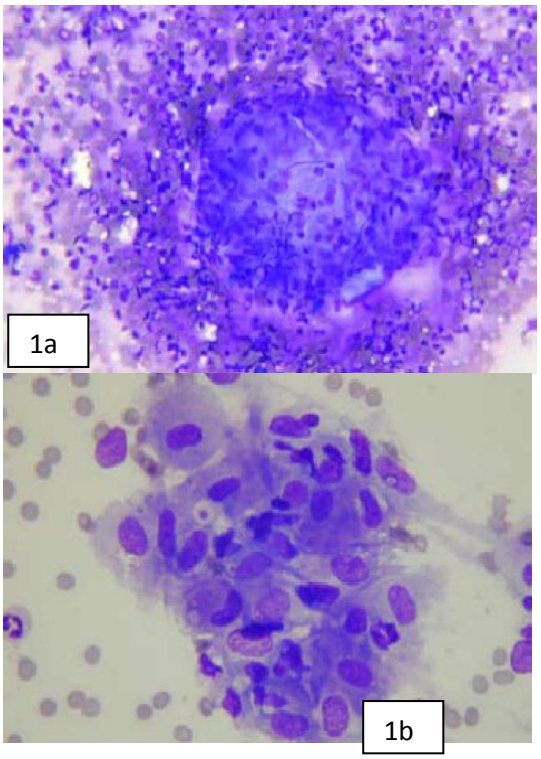

Figure (1a) and (1b): showing granuloma with caseation and epitheliod cells(strong evidence) and without caseation but aggregates of epithelioid cells (weak evidence) by PAP stain.

Table-3 : Distribution of the study population by lymph node site of TB and Its strong or weak evidences.

Sites of Tubercular lymph nodes	Tubercular evidence		Total Number of cases	Percentage
	Strong	Weak		
Cervical	38	18	56	76.71
Submendibular	8	2	10	13.69
Inguinal	5	1	6	8.21
Axillary	1	0	1	1.36
Total	52	21	73	100

On Ziehl Neelson's staining, mycobacterium tuberculosis appeared as red/pink beaded rod-shaped bacteria against a blue background (Figures (2a) and (2b)). There was a association between AFB positivity and caseous necrosis.We have found 38(92%) out of total 41 AFB positive cases with caseous necrosis.We have also found 41 (56%) out of total 73 patients of granulomatous inflammation are positive for AFB.Table-4

Table-4 :Retationship of acid-fast bacilli with caseation necrosis

Caseous necrosis	Acid-fast bacilli		Total Numbers of cases
	Negative for AFB	Positive for AFB	
Not present	18	3(4%)	21
Present	14	38(92%)	52
Total	32	41(56%)	73

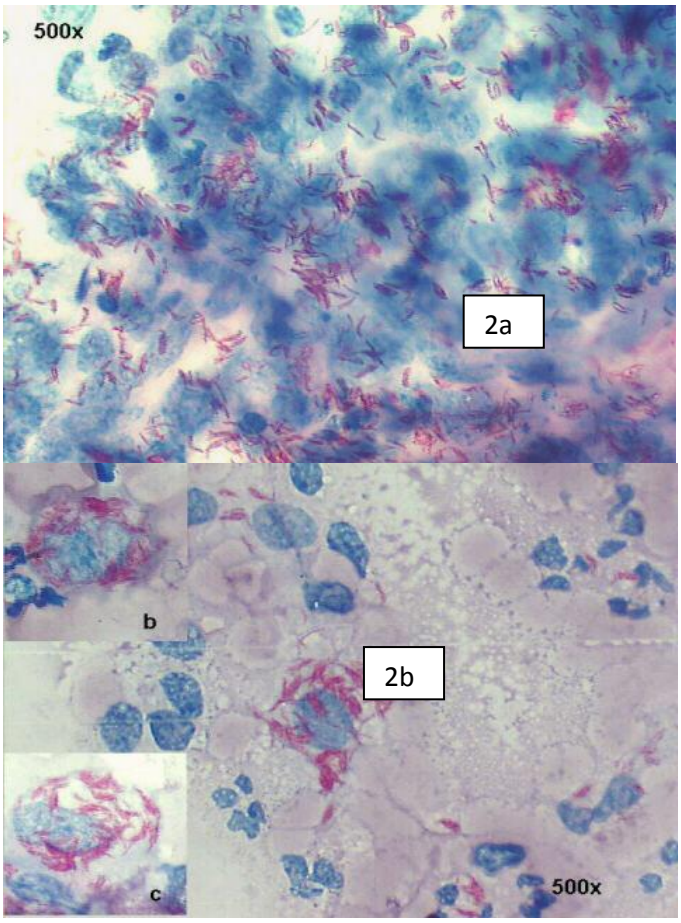

Figure (2a) and (2b):Extracellular and intracellular Mycobaterium Tuberculosis by ZN stain.

In this study, patients age ranges from 6 to 60 years. 45 (61%) patients having tuberculous inflammations were below 30 years of age, shown in Table -5.The male to female ratio was 2.6:1.

Table-5:Distribution of study population by age

Age/Years	Frequency of	Percentage
6-16	19	29.43
17-27	45	61.00
28-38	5	06.84
39-49	2	2.73
50-60	2	2.73
Total	73	100

Discussion

Accurate and timely diagnosis together with effective TB treatment is the mainstay of TB care and control. A confirmed diagnosis of TB can only be given on isolating the M. tuberculosis or finding specific DNA sequence of the bacteria in aspirates. In the resource-poor countries, however, these tests are not within the reach of every individual. In these countries, cost-effective techniques for example, sputum smear microscopy and morphological features are the corner stone of TB diagnosis. In cases of extra pulmonary tuberculosis of lymph nodes, fine needle aspiration cytology (FNAC) is a very useful and reliable test. In areas where tuberculosis is prevalent, diagnosis of TB can be made by seeing the morphological features.

Blind FNAC can approach safely the superficial lesions, including lymph nodes, skin, and soft tissue nodules. In our study, 73 (73%) cases were from lymph nodes are tuberculosis. Many studies have diagnosed TB by aspiration form lymph nodes [16,17,18,19,20].

Cervical lymph node was the most common site of involvement in studies followed by axillary lymph nodes (17,21). Our study was also consistent with above studies in terms of cervical lymph node involvement (69.89%) as the most common anatomic site of granulomatous inflammation. Submandibular lymph nodes were involved in 24.65% cases in our study and was the second most commonly involved site.

Male gender was a slightly more affected (62%) in current study and was in concordance with Bezabih et al. (17). Out of 73 cases, 61% patients in this study were below 30 years of age and 29% were below 20 years. This finding was nearly in accordance with Bezabih et al. in which 69% were below 30 years of age. Based on the facts, it can be inferred that tuberculosis was more commonly seen in young population [17].

Numerous morphological variations in the granulomatous inflammation are seen. There were 52(71.23%) cases with necrosis. The rest 21 (28.76%) of cases were granulomatous inflammation without necrosis. This finding agree with Majeed et al. who found 96% cases with caseation and the rest without.The various morphological presentations of TB have been published (16). International data also supports this variation and studies tried to correlate morphological findings with the AFB staining (17,22).

The Acid Fast Bacilli positivity was labeled after finding red or pink rod-shaped bacteria with beaded appearance (Figures (2a) and (2b)). Regarding AFB positivity variable, results were seen and frequency ranges from 10% to 70% (18 ,23,24,22). In current study, out of 73 cases, 41(56%) cases were positive for AFB. This was in concordance with the international data of a large-scale study of 328 cases, out of which 152 cases (46.4%) were positive for AFB (21). Similarly, our findings agree with Lau et al. who report 47% positivity for tuberculous cases (18) and with Das et al. showing overall 45.8% rate of AFB positivity (25). Some studies report a bit high frequency of AFB positivity. Bezabih et al. reported 59.4% of overall AFB positivity (24), and Vignesh et al. reported 53.3% positivity for single AFB smear (23).

In regions where TB is very common, the morphological findings of granulomatous inflammation is consistent with tuberculosis (24,22). Bangladesh is also included among these countries along with India,Pakistan,Ethiopia, and other African countries. Since epithelioid granulomas, caseation necrosis, giant cells, and AFB positivity are specific for TB, so in these countries excision biopsy can be avoided and antituberculous treatment can be given straightaway [(18).] Excision is not free of complication and is expensive and time consuming, thus it can delay the treatment. Above findings conclude that FNAC with special stains can solely help the physician to start the treatment.

There was an interesting finding in our study.AFB positivity was notably and more commonly found in granulomatous inflammation with caseation necrosis. 38 out of 41 AFB positive cases associated with caseation necrosis (92.68%), in current study. This finding is consistently seen in previous studies (17,21,22). Otherwise in some studies, it is claimed that instead of granulomatous inflammation, if only necrosis or abscess formation is seen, the AFB-positivity increases (18). Dua et al. even documented 100% of AFB positive cases in this scenario (22).

Conclusion:

It is concluded that tuberculous lymphadenitis is still the commonest condition in patients presenting with lymphnode swellings followed by non-specific lymphadenitis and malignant neoplasms especially metastatic carcinoma. FNAC is an easy and suitable tool for the assessment of patients with lymphnodes swellings in the outpatient clinics. Although its diagnostic accuracy is limited as compared to tissue biopsy but it is a good test for both screening and follow-up.The excision biopsy in tuberculous lymph nodes is hazardous since it may cause sinus formation. Therefore, FNAC finding of granulomatous inflammation consistent with tuberculosis and detection of AFB would be very specific and help the physicians to start ATT(anti-tubercular therapy) confidently, immediately as it is cost effective and economical.

References

1. Communicable Disease Department,Tuberculosis.TB in South-East Asia,WHO. Last update: 12 March 2009 .Available from http://www.searo.who.int/en/Section10/Section2097/Section2100_14792.htm
2. A. K. Gupta, M. Nayar, and M. Chandra, "Critical appraisal of fine needle aspiration cytology in tuberculous lymphadenitis," Acta Cytologica, vol. 36, no. 3, pp. 391–394, 1992.
3. Kumar A.Lymph node tuberculosis.In:Sharma SK,Mohan a, editors.Tuberculosis.New Delhi:Jaypee Brothers Medical Publishers; 2001 p. 273-84.
4. .Applied D, Miller HR.Mycobacterial cervical lymphadenopathy: 1981 update. Laryngoscopy 1981;91:1259-66.
5. Thompson MM, Underwood MJ, Sayers RD, Dookeran KA,Bell PRF.Peripheral tuberculous lymphadenopathy: a review of 67 cases.Br J Surg 1992; 79:763-4.
6. World Health Organization (WHO), "Tuberculosis Fact Global and regional incidence," Sheet No. 104-2006: 101.
7. B. Y. Vincent, A. K. William, and H. G. Allan, Blueprints Medicine, Blackwell, 3th edition, 2003.
8 .M. Tekkel, M. Rahu, H. M. Loit, and A. Baburin, "Risk factors for pulmonary tuberculosis in Estonia," International Journal of Tuberculosis and Lung Disease, vol. 6, no. 10, pp. 887–894, 2002.
9. J. M. Grange, "The rapid diagnosis of paucibacillary tuberculosis," Tubercle, vol. 70, no. 1, pp. 1–4, 1989.
10 .Y. S. Shan, J. J. Yan, E. D. Sy, Y. T. Jin, and J. C. Lee, "Nested polymerase chain reaction in the diagnosis of negative Ziehl-Neelsen stained Mycobacterium tuberculosis fistula-in-ano: report of four cases," Diseases of the Colon and Rectum, vol. 45, no. 12, pp. 1685–1688, 2002.
11. N. Kumar, M. C. Tiwari, and K. Verma, "AFB staining in cytodiagnosis of tuberculosis without classical features: a comparison of Ziehl-Neelsen and fluorescent methods," Cytopathology, vol. 9, no. 3, pp. 208–214, 1998.
12. A. M. Lake and F. A. Oski, "Peripheral lymphadenopathy in childhood. Ten-year experience with excisional biopsy," American Journal of Diseases of Children, vol. 132, no. 4, pp. 357–359, 1978.
13 .M. P. Reddy, N. Moorchung, and A. Chaudhary, "Clinico-pathological profile of pediatric lymphadenopathy," Indian Journal of Pediatrics, vol. 69, no. 12, pp. 1047–1051, 2002.

14 .M. M. Goel, V. Ranjan, T. N. Dhole et al., "Polymerase chain reaction vs. conventional diagnosis in fine needle aspirates of tuberculous lymph nodes," Acta Cytologica, vol. 45, no. 3, pp. 333–340, 2001.

15 .J. Glassroth, "Diagnosis of tuberculosis," in Tuberculosis: A Comprehensive International Approach, L. B. Reichmann and E. S. Hershfield, Eds., pp. 149–162, Marcel Dekker, New York, NY, USA, 1993.

16. A. Sarwar, S. Aftab, M. Mustafa, A. Moatasim, S. Siddique, and A. Sani, "Spectrum of morphological changes in tuberculous lymphadenitis," Internation journal of Pathology, vol. 2, no. 2, pp. 85–89, 2004.

17. M. Bezabih, D. W. Mariam, and S. G. Selassie, "Fine needle aspiration cytology of suspected tuberculous lymphadenitis," Cytopathology, vol. 13, no. 5, pp. 284–290, 2002.

18. S. K. Lau, W. U. Wei, C. Hsu, and U. C. G. Engzell, "Efficacy of fine needle aspiration cytology in the diagnosis of tuberculous cervical lymphadenopathy," Journal of Laryngology and Otology, vol. 104, no. 1, pp. 24–27, 1990.

19 .V. Koo, T. F. Lioe, and R. A. J. Spence, "Fine needle aspiration cytology (FNAC) in the diagnosis of granulomatous lymphadenitis," Ulster Medical Journal, vol. 75, no. 1, pp. 59–64, 2006.

20. A. Rajwanshi, S. Bhambhani, and D. K. Das, "Fine-needle aspiration cytology diagnosis of tuberculosis," Diagnostic Cytopathology, vol. 3, no. 1, pp. 13–16, 1987.

21 S. S. Ahmad, S. Akhtar, K. Akhtar, S. Naseem, and T. Mansoor, "Study of fine needle aspiration cytology in lymphadenopathy with special reference to acid-fast staining in cases of tuberculosis," JK Science, vol. 7, no. 1, pp. 1–4, 2005.

22. T. Dua, P. Ahmad, S. Vasenwala, F. Beg, and A. Malik, "Correlation of cytomorphology with AFB positivity by smear and culture in tuberculous lymphadenitis," The Indian Journal of Tuberculosis, vol. 43, pp. 81–84, 1996.

23. R. Vignesh, P. Balakrishnan, E. M. Shankar et al., "Value of single acid-fast bacilli sputum smears in the diagnosis of tuberculosis in HIV-positive subjects," Journal of Medical Microbiology, vol. 56, no. 12, pp. 1709–1710, 2007

24. G. Sethuraman, V. Ramesh, M. Ramam, and V. K. Sharma, "Skin tuberculosis in children: learning from India," Dermatologic Clinics, vol. 26, no. 2, pp. 285–294, 2008.

25. D. K. Das, C. S. Pant, J. N. Pant, and P. Sodhani, "Transthoracic (percutaneous) fine needle aspiration cytology diagnosis of pulmonary tuberculosis," Tubercle and Lung Disease, vol. 76, no. 1, pp. 84–89, 1995.